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Using Phage Lytic Enzymes to Destroy Pathogenic and BW Bacteria

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13. ABSTRACT (Maximum 200 words)

We have exploited the rapid, lethal and highly specific action of bacteriophage lytic enzymes to destroy pathogenic bacteria. Our results show that in vitro 10^7 bacteria can be reduced to sterility seconds after enzyme contact. We now have enzymes that are specific for *S. pyogenes, S. pneumoniae,* and *B. anthracis S. aureus, E. faecalis/E. faecium* and group B streptococci. In animal models, we pre-colonize mice with either streptococcal or pneumococcal species (orally or nasally) and remove them completely with a single dose of phage enzyme delivered to these sites. In a septicemia model with *S. pneumoniae,* bacteria are reduced by >2-logs from the blood of infected animals with a single intravenous dose of enzyme. A lytic enzyme called PlyG from the gamma-phage of *B. anthracis* was specific for all worldwide isolates of *B. anthracis.* When >1 LD100 of *B. anthracis* bacilli were delivered i.v. to mice only 10% of animals, followed for 12 days, survived. When PlyG was injected i.v. 15 min after infection, 90% of the mice recovered fully. Resistance to the enzymes has not been found nor do antibodies neutralize their activity. Thus, phage lytic enzymes are a new reagent to control resistant pathogenic bacteria, offering a capability previously unavailable.

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FINAL REPORT

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Project Goals: We have developed a novel method to kill biowarfare bacteria (particularly *B. anthracis*) safely and quickly. This method exploits the rapid yet specific activity of bacteriophage lytic enzymes to destroy these bacteria on contact. During the course of this grant we planned to develop enzymes that control *B. anthracis*. In preliminary experiments in vitro, 10 micrograms of PlyG lysin from the gamma-phage could reduce the viability of 10^7 bacilli by >6-logs in minutes. In vivo, we were able to save the lives of animals infected intravenously with the Ames strain of anthrax. Our goal was to identify a number of enzymes from a variety of phage to control *B. anthracis*.

Final Report: During the tenure of this grant we were successful in identifying several phage enzymes specific for *B. anthracis*. These enzymes were cloned from the gamma phage (PlyG) and from lysogenic phage found in the *B. anthracis* genome (PlyPH). In animal model experiments, we were able to Infect mice intravenously (iv) with the Ames strain of anthrax and using a single PlyG enzyme dose, save the lives of 70%-90% of the animals whereas 100% of control animals died. Pharmacokenetic experiments revealed that the half-life of these enzymes was about 20 minutes, necessitating the delivery of the enzyme by constant iv infusion to attain maximum effects.

In the presence of a germinating solution, spores will germinate within a minute after exposure. We discovered that in the presence of germinant and lysin, the spore viability could be reduced by >3 logs within 20 minutes. During these studies, we identified a lysin (PlyPH) from a lysogen in the *B. anthracis* genome which retained its lytic activity from pH 4.0 – 8.5. This enzyme has the broadest pH activity range of all reported lysins. We believe that this characteristic and the fact that this enzyme is stable at 60C will be beneficial for its use as a decon enzyme, to remove both spores and vegetative bacilli from the environment and military vehicles.

During the course of this work, we have isolated a number of additional phage that have activity against *B. anthracis*. One of these phage stands out in that we have found that it is more specific for *B. anthracis* than the gamma-phage since it will not recognize the occasional *B. cereus* strain yielding a false positive reaction. This phage is a podoviridae, which is a rare tail-less membrane-containing phage. Because of its high specificity, we believe that this phage may replace the gamma phage a one of the diagnostic tools to identify *B. antheracis*.

We also discovered that when *B. anthracis* becomes lysogenized by its normal phage, that genes that are normally silent are expressed. These genes include, coat proteins, hemolysins, exosporium proteins, lipases and a large number of genes found in the PLCR regulon. The expression of these genes we believe will allow *B. anthracis* to survive in a vegetative state in the soil more successfully that without. This finding has opened the door to a new pathway for *B. anthracis* that was previously missed.

Publications and submitted manuscripts published during the course of this grant:

Schuch, R., D. Nelson and V.A. Fischetti. 2002. Identification of a bacteriolytic agent that can rapidly and specifically detect and kill *Bacillus anthracis.* Nature. 418: 884–889.

Nelson, D., Schuch, R., S. Zhu, D. Tscherne, and V.A. Fischetti. 2003. The genomic sequence of C1, the first streptococcal phage . J. Bacteriol. 185:3325-3332.

Loeffler, J.M, S. Djurkovic and V.A. Fischetti. 2003. The phage lytic enzyme Cpl-1 as a novel antimicrobial for pneumococcal bacteremia and sepsis. Infect. Immun.71:6199-204.

Yoong, P., R. Schuch, D. C. Nelson and V. A. Fischetti. 2004. Identification of a broadly active phage lytic enzyme with lethal activity against antibiotic resistant *Enterococcus faecalis* and *Enterococcus faecium.* **J Bacteriol**. **186**:4808-12.

Cheng, Q., D. Nelson, S. Zhu, and V.A. Fischetti. 2005. Removing group B streptococci colonizing the vagina and pharynx of mice with a bacteriophage lytic enzyme. **Antimicrob Agents Chemother. 49**:111-117.

Schuch, R., V.A. Fischetti. 2005. Complete genome of *B. anthracis* bacteriophages gamma and W and their role in pathogenesis. (Submitted).

Inventors	**Title and RU file number**
1. Fischetti, Vincent A, Schuch, Raymond, Yoong, Pauline, and Nelson, Daniel	PlyPH: a lysin active against anthrax, RU-755
2. Fischetti, Vincent A, Schuch, Raymond, and Yoong, Pauling	Lysins from Enterococcus faecalis RU-654
3. Fischetti, Vincent A. Schuch, Raymond	Lytic Enzymes and spore surface antigens for detection and treatment of B. anthracis bacteria and spores, RU-651
4. Fischetti, Vincent A, Schuch, Raymond, Nelson, Daniel	Phage-associated lytic enzymes for treatment of *B. anthracis* and related conditions, RU-625
5. Fischetti, Vincent A., Loeffler, Jutta	Phage-associated lytic enzymes for treatment of *Streptococcus pneumo*niae and related conditions, RU-621
6. Fischetti, Vincent A, Loeffler, Jutta	Use of synergistic bacteriophage lytic enzymes for prevention and treatment of bacterial infections, RU-629